that the different domain organizations of the two proteins explained their different activities. Thus, Tmod has two tropomyosin (TM)- and two actin-binding sites, organized as: TMBS1-ABS1-TMBS2-ABS2. Lmod is longer, featuring a C-terminal extension that contains a Pro-rich region and a WH2 domain, which constitutes a third actin-binding site. The presence of the WH2 was considered to be the major feature distinguishing Lmod from Tmod. Here we show that this is not the case. Among the main findings are: 1) Lmod not only lacks TMBS2, but also ABS1, such that the entire region N-terminal to ABS2 has very little effect on nucleation, 2) The C-terminal extension of Lmod has also a limited effect on nucleation, and adding it to Tmod produces a very modest increase in nucleation, 3) Despite being relatively well conserved, the major feature distinguishing Lmod from Tmod is ABS2, consistent mostly of a Leu-rich repeat domain. Structural analysis shows that ABS2 can bind up to 3 actin subunits, and subtle differences between Lmod and Tmod dictate the affinities of their interactions with actin, and thus their roles in nucleation vs. capping. Understanding these differences allowed us to engineer an ABS2 Tmod-Lmod hybrid with nucleation activity equal to that of full-length Lmod.

Symposium: Molecular Basis for Mitochondrial Signaling

949-Symp
Systems Approaches to Mitochondrial Calcium Signaling
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Mitochondria are essential hubs of calcium-mediated signaling networks. The organelle can take up, buffer, and release calcium ions to effectively shape intracellular calcium transients, stimulate ATP production and regulate cell death. Although, the basic mechanisms of mitochondrial calcium homeostasis have been firmly established for decades, the molecular identity of the mitochondrial calcium signaling toolkit has evaded classical bottom-up approaches. Our previous studies (1,2) have provided a compelling example of the power of systems approaches applied to mitochondrial calcium signaling to discover hitherto unknown molecular components of the calcium uniporter. Currently, we are developing computational and experimental frameworks for a systematic reconstruction of calcium-dependent signal transduction cascades in mitochondria. By combining evolutionary genomics and loss-of-function genetic and chemical screens, our systems approach holds the potential to shed light on yet unanswered questions in the field of mitochondrial calcium signaling.
1. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK (2011). Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature. 476(7360):341-5.
2. Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK (2010). MICU1 encodes a mitochondrial EF hand protein required for calcium uptake. Nature. 467(7313):291-6.

950-Symp
The Mitochondrial Calcium Uniporter: Molecular Composition and Physiological Role
Rosario Rizzuto.
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Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high $[Ca^{2+}]$ microdomains generated by the opening of ER Ca^{2+} channels. These rapid $[Ca^{2+}]$ changes stimulate Ca^{2+}-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. Ca^{2+} also sensitizes to cell death mediators, e.g. ceramide. In my presentation, I will present the most recent molecular information on MCU, identified by our group in 2011, and the newly identified regulators (MCUb, MICU1, MICU2). I will also show how the availability of molecular tools for MCU now allows to carry out experiments in intact cells and whole organisms that highlight and clarify the importance of mitochondrial calcium homeostasis in physiology and pathophysiology.

951-Symp
Molecular Mechanisms of Mitochondrial Ca^{2+} Uptake: Role of MICU1 and its Paralogs
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Mitochondrial Ca^{2+} uptake is central to cell metabolism, signaling and survival. Recent studies identified MCU as the pore of the mitochondrial Ca^{2+} uniporter and MICU1 as its critical regulator. MICU1 and its paralogs, MICU2 and MICU3 are EF-hand proteins and are the primary candidates to confer Ca^{2+} sensitivity to the Ca^{2+} uniporter. We will present studies of the molecular mechanisms of the MICU-dependent closure of the uniporter at low $[Ca^{2+}]$ levels and its cooperative activation when $[Ca^{2+}]$ increases. Furthermore, we will present clues to the MICU-dependence of the tissue specific mitochondrial Ca^{2+} uptake profiles.

952-Symp
High-Affinity Interaction with VDAC Links Cytosolic Proteins to Mitochondrial Regulation in Health, Cancer, and Neurodegeneration
Tatiana K. Rostovtseva[1], Philip A. Gurnev[2], David P. Hoogerheide[3], Eduardo N. Maldonado[4], John J. Lemasters[5], Olga Protchenko[6], Jennifer C. Lee[7], Sergey M. Bezrukov[1].
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We address the question of how mitochondria control cell survival and cell death by studying the voltage-dependent anion channel (VDAC). VDAC, the major channel of the mitochondrial outer membrane (MOM), is a well-recognized key conduit for ATP and other bioenergetics metabolites fluxes across MOM. We have found that dimeric tubulin, the subunit of microtubule, induces highly efficient reversible blockage of VDAC reconstituted into planar lipid membranes. Although the tubulin-blocked state still conducts small ions, it is virtually impermeable to ATP. We propose that by modulating VDAC permeability for ATP and other respiratory substrates, tubulin controls mitochondrial respiration. These findings are supported by experiments with isolated mitochondria and human hepatoma cells, thus uncovering a mechanism of regulation of mitochondrial energetics by free tubulin and also suggesting how cancer cells preferentially use inefficient glycolysis rather than oxidative phosphorylation (the Warburg effect).
We also found a functional interaction between VDAC and α-synuclein (α-syn), an intrinsically disordered neuronal protein intimately associated with Parkinson disease (PD) pathogenesis. Importantly, in addition to regulation of VDAC permeability by α-syn, our data indicate that VDAC facilitates translocation of α-syn across MOM where it could target complexes of the mitochondrial respiratory chain in the inner membrane. Supporting our *in vitro* experiments, a yeast model of PD shows that α-syn toxicity in yeast depends on VDAC. Considering that VDAC is a major conduit for respiratory substrates across the mitochondrial outer membrane, we conclude that the α-syn/VDAC functional interaction reveals the elusive physiological and pathophysiological roles for monomeric α-syn in PD and also in general neurodegeneration.

Platform: Electron Microscopy and Solution Scattering

953-Plat
GFP for EM: Site-Specific Labeling of Proteins for Electron Microscopy
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Structural analysis of macromolecules by electron microscopy (EM) has been facilitated by a recent technological revolution in instrumentation and data processing, which has led to the achievement of their visualization at atomic resolution. However, moderate resolution electron density maps of protein complexes can be misleading, resulting in ambiguity when ascribing subunits to particular locations within the architecture of complexes. To this end, investigators have traditionally performed subunit mapping by N- or C-terminal fusions with tags, such as maltose binding protein (MBP), with mixed success. Toward the accurate determination of the location and orientation of protein subunits, as well as large scale movements of protein complexes, the establishment of a highly specific labeling technique would be a major breakthrough. Here we present a site-specific labeling strategy that exploits a unique chemical handle introduced by the incorporation of the unnatural amino acid (UAA). The UAA permits a site-specific copper-free click reaction for labeling with a modified label, such as MBP or Nanogold. We use this method to label a subunit

within the recombinant yeast proteasome lid complex that was previously not amenable to labeling by MBP fusion. This technique is extremely versatile and precise; it can be readily expanded to other organisms and to other labels, and will be useful for a wide range of structural analyses.

954-Plat

Fast Shape-Based Global and Local Electron Density Map Search

Juan Esquivel-Rodriguez[1], Xusi Han[2], Charles Christoffer[1], Xuejiao Kang[1], Lyman Monroe[2], **Daisuke Kihara**[3].
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The Electron Microscopy DataBank (EMDB) is growing rapidly, accumulating biological structural data obtained cryo-electron microscopy (cryo-EM). Cryo-EM is an emerging technique for determining large biomolecular complexes and subcellular structures. Together with the Protein Data Bank (PDB), EMDB is becoming a fundamental resource of the tertiary structures of biological macromolecules. To take full advantage of this indispensable resource, the ability to search the database by structural similarity is essential. However, unlike high-resolution structures stored in PDB, methods for comparing low-resolution EM density maps are not well established. Here, we developed a novel computational method for efficiently searching EM maps. The method uses a compact fingerprint representation of EM maps based on the 3D Zernike descriptor, which is a mathematical series expansion for representing isosurface shape of EM maps. The method was implemented in a web server, named EM-SURFER (http://kiharalab.org/em-surfer/), which allows users to search against the entire EMDB with over 2400 entries in a few seconds. By combing with map segmentation, the method can also identify corresponding local regions in EM maps. Examples of search results from different types of query structures are discussed. The unique capability of EM-SURFER to detect 3D shape similarity of low-resolution EM maps should prove invaluable in structural biology.

955-Plat

Cyclophilin a Stabilizes the Mature HIV-1 Capsid through a Novel Non-Canonical Binding Site

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Host cell factor cyclophilin A (CypA) plays an important role in modulating HIV-1 capsid function. Several other host cell proteins, including TRIMCyp and NUP358, contain a CypA domain that interacts directly with viral capsid. The binding of the host protein CypA to the viral capsid is important for infection, yet the structural effects of this binding have not been identified. We determined the cryoEM structure of CypA in complex with an HIV-1 capsid assembly at 8 Å resolution. The density map displays a non-random, selective binding of CypA along the most curved helical direction, forming a bridge directly above the CA CTD-CTD dimer interface cross the adjacent CA hexamers. CryoEM structure-based modeling and large scale all-atoms molecular dynamics simulations reveal unexpectedly that one CypA molecule simultaneously interacts with two CA molecules through a non-canonical novel interface. The individual residuals critical for the interactions were further identified by solution and solid state NMR and confirmed by mutagenesis studies. Our combined cryoEM, computational and NMR studies provide mechanistic insights into the functional role of CypA in modulating capsid uncoating and viral infectivity, and our structure further highlights the novel CypA and CA interface as an attractive therapeutic target for pharmacological intervention.

956-Plat

Residue Specific Radiation Damage of Protein Structures using High-Resolution Cryo-Electron Microscopy

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High-resolution structures of proteins and protein complexes are currently determined using either X-ray crystallography, NMR spectroscopy, or now also cryo-electron microscopy (cryo-EM). The highest resolutions achieved by cryo-EM have been typically restricted to large, well-ordered entities such as helical or icosahedral assemblies or 2D crystals. However, we show that emerging methods in single-particle cryo-EM now allow structure determination at near-atomic resolution, even for much smaller protein complexes with low symmetry. We solved the structure of the ~465-kDa Escherichia coli β-galactosidase at ~3.2-Å resolution using single-particle cryo-EM. At this resolution, the majority of the side-chains, the N-termini, and the geometry of the active sites, including a catalytic Mg2+-ion, can be clearly discerned in the density map. Inspection of the map reveals that while densities for residues with positively charged and neutral side-chains are well resolved, systematically weaker densities are observed for residues with negatively charged side-chains. The negatively charged glutamate and aspartate show on average 30% less density than the similarly sized neutral glutamine and asparagine. This observation is independent of the exposure of these residues to solvent. Analysis of other high-resolution cryo-EM structures reveals similar weaker densities for these types of residues. Radiation damage in X-ray crystallography has been linked to decarboxylation of glutamate and aspartate residues, breakage of disulfide bonds, loss of hydroxyl-groups from tyrosine and methylthio-group of methionine. We now show that negatively charged residues exhibit more pronounced effects of radiation damage in structures solved by cryo-EM. We determined that the degree of damage is dose dependent by comparison of density maps obtained using electron doses ranging from 10-30 electrons/Å2. In summary, we establish the feasibility of determining structures at near-atomic resolution and provide a measure of the effects of radiation damage in high-resolution cryo-electron microscopy.

957-Plat

3D Ultrastructural Investigation of Entire Pancreatic Islets of Langerhans by Serial Block Face Scanning Electron Microscopy

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Serial block-face scanning electron microscopy (SBF-SEM) provides three-dimensional ultrastructure of biological tissues at the nanometer scale (Denk and Horstmann, 2004). Previous studies have applied this technique to visualize the architecture of pancreatic islets of Langerhans in wild-type mice. Initial results indicate that an average beta cell has almost twice the cellular volume of an average alpha cell and four times the mitochondrial volume, whereas the nuclear volumes in both cell types are approximately equal. Comparisons of insulin-secreting beta granules and glucagon-secreting alpha granules show that the beta granules have more pronounced halos and diameters twice that of the alpha granules. In addition, three-dimensional rendering of islet blood vessels reveals that all secretory cells in an islet are in contact with the pericapillary space with an average contact area of 9% +/− 5% of the plasma membrane's surface. We are currently applying SBF-SEM to determine quantitatively how islet morphology differs between genotypes in wild type, non-obese diabetic (NOD) and IA-2/IA-2 beta double-knockout mice. Such quantitative analysis of ultrastructural differences between controls and diabetes-related animal models could help further understanding of disease. The research was supported by the intramural programs of NIBIB and NIDCR.

Denk, W., & Horstmann, H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS biology, 2(11), e329.

958-Plat

Superresolution Fluorescence Microscopy within a Scanning Electron Microscope

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Correlative optical and electron microscopy has the potential to provide detail on the organization of biological static structures at multiple length scales, but conflicting technical requirements on experimental design, molecular identification, and sample preparation present major obstacles. While fluorescence microscopy is usually impossible in an electron microscope, we present nondestructive fluorescence imaging of biological samples in an SEM. Our novel chamber allows a scanned electron beam to indirectly excite fluorescence with nanometer-scale resolution while simultaneously protecting the sample